

decreases thinning out time, keeping thinning out time more consistent (~30min). Lipid solution was deposited in a small aperture fabricated on a PDMS film and was frozen before use. Upon thawing a lipid bilayer is spontaneously reconstructed with the mean formation time of ~30 min with high success rate (>80%). We also show potential applications with lipid bilayers created in a PDMS film due to its versatility

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Noise Properties of Ion Current in Rectifying Nanopores

Matthew Powell, Ken Healy, Matt Davenport, Sa Niya, Lane Baker, Sonia Letant, Zuzanna Siwy.

Studying noise properties of ion currents in nanopores can improve detection limits for nanopore sensors as well as give insight into behavior of transport at the nanoscale. We focused on the $1/f$ noise that is observed in the low frequency regime of the ion current power spectra with the exponent $\alpha \sim 1$. We found that $1/f$ noise in single conically shaped nanopores in polymer films and glass nanopipettes exhibits asymmetric noise properties with respect to voltage polarity which are not observed for cylindrical and silicon nitride nanopores. The noise asymmetry is shown by the normalized power spectra, which present the noise amplitude at a given frequency, typically 1 Hz for these measurements, divided by the ion current squared. The conically shaped structures rectify the ion current and the currents for the forward bias exhibit noise that increases with voltage in an exponential manner, and are weakly KCl concentration dependent. The normalized noise of currents in the reverse bias is typically voltage-independent but increases with the increase of KCl concentration. The difference in noise properties of the currents is most pronounced when the pore diameter is comparable to the thickness of the electrical double-layer. We discuss two models, which could explain the observed effects: (i) presence of air bubbles, and (ii) crowding of ions at the pore entrance.

Fluorescence Spectroscopy III

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Tryptophan Fluorescence from G. Weber to the Present Ludwig Brand.

Gregorio Weber (Symposium on Light and Life, W.D. McElroy and Glass, B. Eds., 1961) described the fluorescence of tryptophan in proteins. Since that time more than 12,000 papers have appeared related to tryptophan fluorescence. It is used to measure the folding of proteins and the interaction of proteins with each other, with nucleic acids, membranes and small molecules. Time-resolved fluorescence studies have revealed details regarding the excited-state of tryptophan in proteins. Ground-state and excited-state heterogeneity influence the fluorescence and its relation to protein conformation. Excited-state electron transfer, proton transfer, energy transfer, solvent and protein relaxation are among the processes that have been implicated in tryptophan fluorescence in proteins. The availability of new experimental, computational and theoretical methods suggest that there are now opportunities for using tryptophan fluorescence for probing protein structure and dynamics. Femtosecond time-resolved techniques and molecular dynamics computations have been of particular value and should provide new information about folded and unfolded structural regions in proteins.

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Protein Hydration and Coupled Water-Protein Fluctuations Probed by Tryptophan

Dongping Zhong.

Using tryptophan with site-directed mutagenesis, we can map out global hydration dynamics and water-protein fluctuations with femtosecond resolutions. We clearly demonstrated that tryptophan is a powerful optical probe to study protein hydration.

3295-Pos Board B400

TDSS in Trp Fluorescence Reveals Multiple Protein and Solvent Relaxation Modes

Dmitri Topygin, Thomas B. Woolf, Ludwig Brand.

The Time-Dependent Spectral Shifts (TDSS) in the fluorescence of solvatochromic dyes in polar solvents report solvent relaxation dynamics, which in water occurs on the femtosecond timescale. The TDSS in the emission of tryptophan and other solvatochromic fluorophores in proteins span a range of time-scales from femtoseconds to nanoseconds. MD simulations of the GB1 protein in TIP3P water made it possible to separate five relaxation modes and to explain their physical origins. Two of these relaxation modes also contribute to the TDSS of dyes in polar solvents.

The ultrafast relaxation mode ($\tau \sim 35$ fs) is due in part to the librational relaxation of water molecules and in part to the small adjustments in the local protein structure. This mode is responsible for about half of the total TDSS amplitude.

Two collective rotational relaxation modes of water molecules are known. The longitudinal ($\tau_L \sim 550$ fs) mode contributes to the TDSS of both dyes in water and tryptophan residues in proteins. The transverse ($\tau_D \sim 8.3$ ps) relaxation cannot contribute to the TDSS of dyes in water, but it contributes to the TDSS in proteins having internal water channels or pockets. This can be used to study internal water. In MD simulations using TIP3P water both τ_L and τ_D are ~30% shorter than the experimental values.

A small (<0.25 Å) adjustment of the GB1 tertiary structure occurs in MD simulations on the time scale of 130ps and results in a 140cm^{-1} contribution to the TDSS. A shift in the sidechain conformation of Glu-42 in close proximity to the fluorophore (Trp-43) is the main contributor to the slow TDSS amplitude (470cm^{-1}). This conformational change takes 2.6ns in MD simulations and only 80ps in the experiment, which reveals that in CHARMM22 the potential barriers separating sidechain conformations are too high.

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Nonradiative Processes in Constrained Trps and Model Compounds

Mary D. Barkley.

Constrained derivatives and model compounds were used to elucidate the non-radiative decay pathways of Trp. Fluorescence quenching by electron transfer from the excited indole to the amide backbone was studied in 7 cyclic hexapeptides.

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5-Fluorotryptophan as Fluorescent Probe to Characterize an Oligomeric Membrane Protein

Jaap Broos.

The mannitol transporter from *E. coli*, EII^{mtl}, belongs to a class of membrane proteins coupling the transport of substrates with their chemical modification. EII^{mtl} is functional as a homodimer and it harbors one high-affinity mannitol binding site in the membrane-embedded C domain (IIC^{mtl}). To localize this binding site, single Trp containing mutants of EII^{mtl} were mixed with azi-mannitol, a substrate analogue acting as a Förster resonance energy transfer (FRET) acceptor (R_0 of 10 Å). Due to the complex fluorescence decay of Trp, we could not establish whether one or both Trp residues showed FRET with azi-mannitol. To overcome this, we took advantage of the homogeneous decay of 5-fluorotryptophan and this analog was biosynthetically incorporated in 19 mutants. Typically, for mutants showing FRET, only one 5-FTrp was involved, while the 5-FTrp from the other monomer was too distant. This proves that the mannitol binding site is asymmetrically positioned in dimeric IIC^{mtl}. The FRET results localized the position of the binding site halfway the first transmembrane helix. Combined with available 2D projection maps of IIC^{mtl}, it is concluded that a second resting binding site is present in this transporter. This work demonstrates the potential in structural and mechanistic protein research of a donor-acceptor pair with a very short R_0 , of which the donor shows homogeneous fluorescence decay kinetics.

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Time Resolved Fluorescence of the Single Tryptophan in R61, a DD-Carboxypeptidase from Streptomyces: Contributions of Dynamics and Heterogeneity

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The fluorescence emission of the single tryptophan (W233) of the mutant protein DD carboxypeptidase from *Streptomyces* is characterized by a red edge excitation shift (REES). This phenomenon is an indication for strongly reduced dynamics in the environment of the tryptophan residue, which has a very low accessibility to the solvent. The Stokes shift however, shows an unusual temperature and time dependence. This, together with the fluorescence lifetime analysis, showing three resolvable lifetimes, can be explained by the presence of three rotameric states which can be identified using the Dead End Elimination (DEE) method. The three individual lifetimes increase with increasing emission wavelength. This is interpreted as each individual lifetime being an average lifetime on its own, indicating the presence of restricted protein dynamics within the rotameric states. This is confirmed by time resolved anisotropy measurements, which demonstrate dynamics within the rotamers but not among the rotamers and by maximum entropy analysis producing distributions that shift with the emission wavelengths. The maximum entropy distributions can also be fitted using a gamma-function analysis, again indicating a dynamic component next to a static heterogeneity. Advanced DEE calculations together with MD simulations indicate the existence of two minima (i.e. substates) within one particular rotamer, with frequent transitions. The global picture is that of a protein with a single buried tryptophan showing strongly restricted dynamics within three distinct rotameric states, one of which is further subdivided into two substates, with different emission spectra.